Fig. 2 illustrates this for the case of NEM. Some 30–40 min after application of this sulfhydryl reagent (10^{-5}–10^{-4} M), a decrease in junctional membrane conductance is apparent. Cell depolarization, on the other hand, develops without delay. At higher concentrations of NEM (up to $5 \cdot 10^{-3}$ M, the highest used), the change in junctional conductance develops sooner and at a time when cell resting potential is still fairly high (up to 45 mV). With concentrations of NEM ranging down to about 10^{-5}–10^{-4} M the conductance change is not reversed by washing the cell system in control medium. Effects essentially similar, both in intensity and in time course, were obtained with 2,4-dinitrophenol (10^{-4} M).

Ouabain (4 experiments) caused no significant effects on junctional membrane conductance during 2 h of application, even at the relatively high concentration of 10^{-4} M. The cells, however, depolarize and the non-junctional conductance rises by a factor of 2 to 7. Sodium azide (10^{-3} M) (2 experiments) produced no detectable reduction in junctional membrane conductance in 1 h.

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Evidence for a special type of bicarbonate transport in the isolated colonic mucosa of *Bufo arenarum*

The ability of the colonic mucosa to transport Na^+, *in vivo* and *in vitro*, from mucosa to serosa, against its electrochemical gradient, has been demonstrated in mammalia (CURRAN AND SCHWARTZ[1], COOPERSTEIN AND BROCKMAN[2]) and amphibia (USSING AND ANDERSEN[3], COOPERSTEIN AND HOGBEN[4]). In *Rana catesbeiana*[4], the Na^+ transport does not explain the whole short circuit current and the ionic species responsible for the difference observed has not been identified. The present report demonstrates the existence of at least two different components of the short circuit current in the isolated colonic mucosa of the South American toad *Bufo arenarum*.

Experimental evidence will be presented suggesting that part or all of the non-sodium component of the short circuit current is explained by a special type of HCO_3^- transport compatible with the lack of a radioactive net flux, in the short circuit state.

Experiments were performed on female *Bufo arenarum*, weighing between 250 and 350 g, within the first 2 weeks of captivity. During this period the observed difference between the short circuit current and the Na^+ net flux was maximal. The animals were placed under light ether anesthesia. Immediately afterwards, the colon was separated from the animal through a ventral incision, and the muscular layer dissected from the mucosa, following the technique of PARSONS AND PATERSON[5]. The colonic sac was opened, and two independent portions of the same membrane were mounted between two lucite chambers having an area of 1 cm^2, as described by USSING AND ZERAHN[6], with similar devices for measurement of the potential difference and short circuit current . Unless otherwise stated, the composition of the Ringer fluid was (in mM): Na^+, 110; K^+, 2.5; Cl^-, 79.5; HCO_3^-, 30; $H_2PO_4^-$, 0.33; HPO_4^{2-}, 1.33; SO_4^{2-}, 0.5; Ca^{2+}, 1.25; gluconate, 2.5; glucose, 5.5.

The radioactive unidirectional fluxes were measured in each direction, each one in a separate portion of a single colon. The short circuit current and Na^+ flux values obtained on colonic mucosa dissected from animals that were in the first 2 weeks of captivity showed a marked dispersion. Therefore, to standardize the values and allow statistical analysis of paired data, they were calculated as the fraction of the short circuit current due to the Na^+ net flux (Na^+ net flux per short circuit current). The Na^+ net flux was calculated as the difference between the unidirectional fluxes measured in the two independent portions of the same colon and the short circuit current as the average of the 3 to 6 values obtained in each experimental flux period. Using this procedure, the isotopic fluxes of ^{22}Na, ^{36}Cl and ^{14}C of the HCO_3^- were measured in the short circuit current. After allowing 1 h of equilibration in the solution which contained the labeled ion, samples were withdrawn every 30 or 60 min during two to four periods. ^{22}Na was measured with a crystal scintillator, ^{36}Cl with a Geiger tube and ^{14}C-labeled bicarbonate, previously precipitated as $BaCO_3$, with a low-background gas-flow counter. A minimum of 10 000 counts/min were counted in each sample.

COOPERSTEIN AND HOGBEN[4] reported that the absence of HCO_3^- and CO_2 in the bathing solutions, at constant pH, lowers the potential difference and short circuit current sharply. In a previous report[7], the effect of the individual components of the HCO_3^-–CO_2 system on each side of the membrane, was determined. The results demonstrated that the potential difference and short circuit current were depressed only by the absence of HCO_3^- in the serosal bathing solution. On the other hand, replacing the 5% CO_2–95% O_2 gas mixture by 100% O_2, in the presence of 30 mM HCO_3^- in the serosal solution, markedly raises the short circuit current and potential difference. Neither the pH change alone, nor the absence of HCO_3^- on the mucosal side, affected these parameters.

The Ringer solutions used in the present experiments were gased with 100% O_2. Under these conditions, the concentration of CO_2 of the normal Ringer was negligible. The measured flux of the ^{14}C isotope was, therefore, considered as the HCO_3^- flux. The relative error introduced by the presence of traces of CO_2 in the flux calculations was less than 1%, as computed from the concentration of CO_2 calculated with the

Henderson–Hasselbach equation, and even assuming free diffusion of CO_2 through the membrane ($D = 2.8 \cdot 10^{-5}$ cm²/sec)[9].

The colonic mucosa of *Bufo arenarum* exhibited a spontaneous potential difference averaging 35 mV, with the serosa positive relative to mucosa. These values decreased with the time of captivity and fasting of the animals. The Na^+

TABLE I

Na^+, Cl^- AND HCO_3^- FLUXES AND SHORT CIRCUIT CURRENT IN ISOLATED COLONIC MUCOSA OF *Bufo arenarum*

The results are expressed as mean ± S.E.

Ion	*Mucosa–serosa flux*	*Serosa–mucosa flux*	*Mean of the net fluxes*	*Short circuit current*	*% of the short circuit current due to the Na^+ net flux*
	(μequiv /cm² · h)				
Na^+ (6,14)*	1.92 ± 0.20	0.21 ± 0.04	1.71 ± 0.21	2.46 ± 0.31	70.6 ± 6.7 $P < 0.01$ paired data
Cl^- (5,11)	1.03 ± 0.11	0.97 ± 0.11	0.06 ± 0.08	3.13 ± 0.28	
HCO_3^- (4,7)	0.71 ± 0.08	0.80 ± 0.11	−0.09 ± 0.07	2.11 ± 0.17	

* First numbers in parentheses indicate the amount of experiments and the second ones the total number of experimental periods.

net flux (see Table I) represented on average 70.6% ± 6.7 S.E. ($P < 0.01$, paired data) of the short circuit current. No net flux significantly different from zero was found for either Cl^- or HCO_3^- (0.06 ± 0.08 S.E. and −0.09 ± 0.07 S.E., respectively).

In another set of experiments, 8 colonic sacs were dissected with the same technique and incubated for 4 h at room temperature. The mucosal side was bathed with 2 ml of free bicarbonate Ringer (30 mM NaCl instead of 30 mM $NaHCO_3$), previously saturated with 100% O_2. The serosal side was bathed with 100 ml of normal Ringer with ^{14}C-labeled bicarbonate with a specific activity of 19 μC/mmole and saturated with 100% O_2. This Ringer solution acted as a reservoir. Six other experiments were performed with everted colonic sacs. The 30 mM bicarbonate Ringer bathed the mucosal surface and the free bicarbonate Ringer the serosal one. At the end of the incubation period, in order to calculate the specific activity of the HCO_3^- inside the sacs, the following parameters were measured: final mucosal volume, determined gravimetrically; final mucosal HCO_3^- concentration, determined with a Van Slyke manometric apparatus; and the internal and external radioactivity, measured in a fashion similar to that described for the flux experiments.

A dilution of the specific activity of HCO_3^- was observed, whose magnitude indicated that a fraction of 41.0% ± 3.1 S.E. of the HCO_3^- present inside the sacs, at the end of the incubation period, had an intracellular origin. The final internal HCO_3^- concentration averaged (in mM) 11.2 ± 2.2 S.E. In the everted sacs the dilution of specific activity of HCO_3^- was a fraction of 1.5 ± 0.9 S.E. % of the

HCO_3^- present inside the sacs at the end of the incubation period. The final internal HCO_3^- concentration averaged (in mM) 7.3 ± 1.6 S.E.

According to the previous results the Na^+ transport, by itself, does not account for the total short circuit current observed in this mucosa. There is a fraction of about 30% of the short circuit current that can only be explained on the basis of an anion transport from serosa to mucosa or a cation transport in the opposite direction.

The results allow the exclusion of Cl^- and apparently also of the HCO_3^-. The observed dilution of the specific activity of the HCO_3^- on the mucosal side, indicated that this ion is transferred from the intracellular space of the membrane to the mucosal side. This fact can only be reconciled with the absence of a HCO_3^- net flux, in the short circuit current, if it is assumed that the intracellular HCO_3^- does not equilibrate in specific activity with the labeled HCO_3^- present in the external solutions. HCO_3^- could arise from CO_2 metabolically formed in the cell[8]. Once formed, this HCO_3^- could then be transferred to the mucosal side, participating in the short circuit current. Although these results clearly demonstrate the existence of a HCO_3^- transport, no quantitative evaluation of its participation in the short circuit current can yet be made. This transport cannot be detected by the classic method of studying its isotopic net flux in the absence of electrochemical gradients. The present findings do not allow one to conclude whether the primary process is the passage of HCO_3^- from the cell to the mucosal side or of H^+ in the opposite direction.

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